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IN THE DIGESTION OF GELATIN. BY R. H.
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Reprinted from the Journal of Physiology.

Vol. XII. No. 1, 1891.

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THE PRIMARY CLEAVAGE PRODUCTS FORMED IN
THE DIGESTION OF GELATIN. BY R. H. CHITTEN-
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(Contributions from the Sheffield Biological Laboratory
of Yale University.)

It has long been known that gelatin, under the influence of gastric and pancreatic juice, becomes liquid and loses entirely the power of gelatinization. As early as 1826 Tiedemann and Gmelin¹ made this observation with natural gastric juice, and later Metzler², Schweder³, Etzinger⁴ and others obtained the same result with artificial gastric juice. Similar observations were likewise made by Schweder, Nencki, and Kühne, with pancreatic juice.

Obviously, under these circumstances the gelatin underwent some kind of change, but whether it consisted simply in a conversion of the substance into a soluble and non-gelatinous modification, or of a cleavage into two or more widely different bodies, was not known.

The most important investigation bearing upon this point is the well-known work of Hofmeister⁵, in which he found that solutions of gelatin subjected to the action of boiling water for a longer period than 25 hours, lost the power of gelatinizing, being converted through hydration into a mixture of two peptone-like bodies, to which the names of semiglutin and hemicollin were given. These two so-called gelatin-peptones were unlike each other in chemical composition and reactions, and furthermore differed widely in composition from the gelatin-peptone obtained by Nencki⁶ in the decomposition of gelatin

¹ *Die Verdauung nach Versuchen*, Heidelberg, 1826, Bd. i. p. 171.

² *Beiträge zur Verdauung des Leims*, Giessen, 1860. Schmidt's *Jahrb.*, Bd. 110, p. 153.

³ Virchow's *Jahresbericht*, 1867, Bd. i. p. 152.

⁴ *Zeitschrift für Biologie*, Bd. x. p. 92.

⁵ "Ueber die chemische Structur des Collagens." *Zeitschrift für physiologische chemie*, Bd. ii. p. 299.

⁶ "Ueber die Zersetzung der Gelatine und des Eiweisses bei der Fäulniss mit Pancreas." Bern, 1876. Abstract in *Jahresbericht für Thierchemie*, Bd. vi. p. 31.



with the pancreatic ferment and by putrefaction. Nencki's products contained 40—41 per cent. of carbon and 15.27—15.46 per cent. of nitrogen, thus showing a wide divergence in composition from gelatin itself, and indicating a decomposition of considerable magnitude.

With elastin, experiments carried out in this laboratory¹ have shown that the products [elastoses] formed in the gastric and pancreatic digestion of this albuminoid do not differ in composition from the mother substance to the extent usually found with the genuine proteids. To be sure, deuteroelastose does contain a somewhat lower percentage of carbon than elastin itself, a fact which accords with what has usually been found true of deuteroproteoses in general, but in this particular case the diminution is not large. It is in fact very questionable whether, in the digestion of an albuminoid, the chemical changes which take place are of the same degree or magnitude as in the case of true proteids. With the latter bodies, repeated experiments have shown that the corresponding proteoses and peptones are formed by a gradual series of hydrations, of which the gradually diminished percentage of carbon in the products is a good indication. With the albuminoid elastin, on the other hand, the changes in the content of carbon in the various cleavage products are far less marked, although the changes are undoubtedly of a hydrolytic nature. It seemed desirable therefore to study the products formed in the digestion of the related albuminoid gelatin, especially as the study of this body adds one more to the list of proteids and albuminoids, the digestion products of which have been carefully studied, thus bringing us so much nearer to the point from which eventually we hope to be able to draw new and more definite generalizations regarding the chemical changes produced by the digestive ferments.

Gelatin, its preparation and composition.

The gelatin used in the following experiments was prepared by soaking several pounds of the best French gelatin in cold distilled water, repeatedly changed, for several days until all soluble salts were removed. The swollen mass was then heated gently until dissolved, and the gelatin precipitated by pouring the warm fluid slowly into a large volume of 93 per cent. alcohol. The tough, stringy precipitate was strained off, placed under pressure to remove as much of the alcohol as possible, and then warmed gently for some time to expel

¹ See *Zeitschrift für Biologie*, Bd. xxv. p. 368.

the last traces of alcohol. Of this product a small sample was further treated, preparatory to analysis, by dissolving it in hot water, filtering the solution through paper with the aid of a hot water funnel, then evaporating the solution to dryness on a water-bath until it was quite hard and brittle. It was finally ground to a fine powder and dried at 110°C . until of constant weight.

Analysis of Gelatin.

- I. 0.2590 gram substance gave 0.1578 gram H_2O = 6.76 per cent. H and 0.4639 gram CO_2 = 48.84 per cent. C.
- II. 0.2969 gram substance gave 0.1770 gram H_2O = 6.73 per cent. H.
- III. 0.2704 gram substance gave 0.1631 gram H_2O = 6.70 per cent. H and 0.4827 gram CO_2 = 48.68 per cent. C.
- IV. 0.3145 gram substance gave 45.6 c.c. N at 7.2°C . and 758.3 mm. pressure = 17.70 per cent. N.
- V. 0.2843 gram substance gave 41.4 c.c. N at 7.0°C . and 760.2 mm. pressure = 17.80 per cent. N.
- VI. 0.5439 gram substance gave after fusion with $\text{KOH} + \text{KNO}_3$ 0.0264 gram BaSO_4 = 0.66 per cent. S.
- VII. 0.4563 gram substance gave after fusion with $\text{KOH} + \text{KNO}_3$ 0.0257 gram BaSO_4 = 0.75 per cent. S.
- VIII. 0.4025 gram substance gave 0.0051 gram ash = 1.26 per cent.
- IX. 0.3765 gram substance gave 0.0048 gram ash = 1.27 per cent.

Percentage composition of the ash-free substance.

								Average
C	49.46	—	49.30	—	—	—	—	49.38
H	6.84	6.81	6.78	—	—	—	—	6.81
N	—	—	—	17.92	18.02	—	—	17.97
S	—	—	—	—	—	0.67	0.76	0.71
O								25.13
								<hr/> 100.00

As seen from the analysis, the composition of the dried gelatin differs slightly from the composition of gelatin as usually published, in that it contains a somewhat smaller percentage of carbon. Thus Gorup-Besanez¹ gives the composition of gelatin from tendons as C 50.2, H 6.7, N 17.9, O + S 25.0. Another analysis of our product, however, gave essentially the same results as those quoted above, from

¹ *Lehrbuch der physiologischen chemie.* Dritte Auflage, 1874, p. 150.

which we must conclude that the analysis rightfully represents the composition of the sample of gelatin used in our experiments.

Treatment of gelatin with pepsin-hydrochloric acid.

600 grams of purified and partially dried gelatin were warmed at 40° C. with 2½ litres of a pure and active pepsin-hydrochloric acid solution, the acid being of 0.4 per cent. strength. The digestion was continued for three days, at the end of which time there remained only a small amount of insoluble matter. This antialbumid-like substance was heavy rather than flocculent, and was at least partially soluble in 0.5 per cent. sodium carbonate. Addition of hydrochloric acid to the alkaline solution gave rise to a permanent precipitate only when the acid was present in slight excess; the precipitate so produced was soluble in a large excess of acid. The substance gave the ordinary proteid reactions with Millon's reagent, also with the biuret and xanthoprotein tests. Unlike the antialbumid formed in the gastric digestion of most proteids, it was not affected by treatment with an alkaline solution of trypsin at 40° C. It contained a large amount of ash.

The clear yellow filtrate from the undigested residue containing the products of digestion was neutralized, without yielding any neutralization precipitate, and then concentrated to a syrup. The syrupy solution on cooling showed no signs whatever of gelatinizing. On saturating the fluid, while hot, with ammonium sulphate, a heavy gummy precipitate resulted, composed of the primary products of digestion, the gelatoses, while in the filtrate there remained a small amount of true gelatin-peptone, non-precipitable by saturation with ammonium sulphate. The latter, however, was present in such small amount that it could not be separated from the ammonium sulphate in sufficient quantity for analysis.

Examination of the ammonium sulphate precipitate revealed the presence of two distinct bodies; one, corresponding to protoalbumose and like it precipitated in part by saturation with salt, and completely on addition of a little acetic acid to the salt-saturated fluid; the other, corresponding to deutoalbumose and like the latter precipitated by saturation with ammonium sulphate, after the proto body had been completely removed from the fluid.

The gelatoses precipitated collectively by saturation with ammonium sulphate were purified somewhat by solution in water and

re-precipitation with the ammonium salt, after which they were again dissolved in water and the proto and deuterogelatose separated from each other.

Protogelatose.

All protoproteoses thus far studied have been partially precipitated by saturation of their neutral solutions with sodium chloride, and completely precipitated on addition of a little acetic acid to the salt-saturated fluid. Protogelatose, however, seemed characterized by very incomplete precipitation with salt alone. Saturation of the neutral fluid with sodium chloride gave only a comparatively small amount of precipitate. The substance, however, was completely precipitated by addition of 30 per cent. acetic acid to the salt-saturated fluid, and apparently little, if any, deuterio was thrown down at the same time. The reactions of that portion of the gelatose precipitated by salt alone agreed exactly with those of the portion thrown down on addition of salt-saturated acetic acid. Further, the percentage of carbon and nitrogen was the same in the two portions, and as the amount of substance precipitated by salt alone in a perfectly neutral fluid was comparatively small, it seemed probable that the portion thus precipitated was simply a trace of the body thrown down so abundantly on adding a few drops of acid to the salt-saturated fluid.

Precipitated in this manner, by salt and acid, protogelatose came down as a heavy gummy mass, soluble in excess of acid, which by standing took on the appearance of a thick, yellowish-brown oily gum. It was purified by being dissolved in water, the solution saturated with pure salt, by which more or less of the substance was precipitated, and finally completely precipitated by addition of a little salt-saturated acetic acid. The substance was then again dissolved in water, the solution carefully neutralized with sodium hydroxide and dialyzed in running water until all soluble salts were removed, after which the filtered fluid was concentrated to a thick syrup and the body precipitated by strong alcohol. After thorough washing with alcohol, the precipitate was dissolved in a little water, with the aid of heat, and the solution dried on a water-bath until the substance appeared hard and brittle. It was then ground to a powder and dried at 110° C. until of constant weight.

The composition of the body is shown in the following results :

Protogelatose, gastric digestion.

- I. 0.6025 gram substance gave 0.3646 gram H_2O = 6.72 per cent. H and 1.0828 grams CO_2 = 49.00 per cent. C.
- II. 0.2947 gram substance gave 0.1747 gram H_2O = 6.58 per cent. H and 0.5294 gram CO_2 = 48.98 per cent. C.
- III. 0.2917 gram substance gave 42.4 c.c. N at 8.3° C. and 750.1 mm. pressure = 17.45 per cent. N.
- IV. 0.2800 gram substance gave 41.0 c.c. N at 13.0° C. and 761.1 mm. pressure = 17.56 per cent. N.
- V. 0.6500 gram substance gave after fusion with KOH + KNO_3 0.0271 gram $BaSO_4$ = 0.57 per cent. S.
- VI. 0.4525 gram substance gave after fusion with KOH + KNO_3 0.0154 gram $BaSO_4$ = 0.46 per cent. S.
- VII. 0.5732 gram substance gave 0.0117 gram ash = 2.04 per cent.
- VIII. 0.5166 gram substance gave 0.0100 gram ash = 1.93 per cent.

Percentage composition of the ash-free substance.

							Average
C	49.99	49.97	—	—	—	—	49.98
H	6.85	6.71	—	—	—	—	6.78
N	—	—	17.83	17.89	—	—	17.86
S	—	—	—	—	0.58	0.47	0.52
O							24.86
							100.00

From these results it is evident that the proto body does not differ materially in composition from gelatin itself. Like other protoproteoses it is to be considered as one of the primary products of digestion with pepsin-acid, and like them it is capable of being further converted into true peptone by the long continued action of powerful pepsin solutions. Thus in one experiment, where a given weight of pure protogelatose was warmed for 137 hours at 40° C. with a pure and strong pepsin-acid solution, 20 per cent. of the body was converted into peptone, non-precipitable by ammonium sulphate.

With Millon's reagent, an aqueous solution of protogelatose gives a slight white precipitate, soluble in excess of the reagent. Heated, the fluid takes on a bright pink colour, but no precipitate is produced on boiling, unless very concentrated, when a pink coagulum appears.

Cupric sulphate gives no precipitate; addition of potassium hydroxide, however, brings out a violet red colour.

Tannic acid gives a heavy, curdy white precipitate.

Chloroplatinic acid gives a heavy, yellow precipitate, readily dissolved by warming and reappearing as the solution cools.

Picric acid likewise produces a heavy yellow precipitate, which dissolves as the solution is warmed, reappearing in the cold.

Mercuric chloride gives a heavy flocculent precipitate, which disappears in part on warming the mixture, reappearing on cooling.

Like other protoproteoses the substance is readily soluble in cold water.

Deutero-gelato-se.

This substance is obtained by treating the original filtrate from the proto body, with ammonium sulphate. The separation is preceded by the removal of the sodium chloride from the salt-saturated fluid. This is very essential, as otherwise the precipitation by ammonium sulphate will be very incomplete or may fail altogether. To separate the salt considerable strong alcohol was added to the salt-saturated acid fluid, the salt crystals filtered off, the filtrate concentrated and finally dialyzed for several days until silver nitrate gave little or no reaction. The solution was then concentrated somewhat and saturated, while hot, with ammonium sulphate. As a result a heavy, stringy gummy precipitate was obtained. The deutero body thus precipitated was redissolved in water and again precipitated by the ammonium salt, after which it was once more dissolved in water and dialyzed until entirely free from sulphate. From the filtered fluid, after concentration to a syrup, the deutero body was precipitated by alcohol, and finally dried at 110°C . until of constant weight.

On analysis the following results were obtained:

Deutero-gelato-se, gastric digestion.

- I. 0.4091 gram substance gave 0.2490 gram H_2O = 6.76 per cent. H and 0.7299 gram CO_2 = 48.65 per cent. C.
- II. 0.2924 gram substance gave 0.1785 gram H_2O = 6.78 per cent. H and 0.5230 gram CO_2 = 48.77 per cent. C.
- III. 0.2978 gram substance gave 43.1 c.c. N at 13.6°C . and 758.6 mm. pressure = 17.28 per cent. N.
- IV. 0.2814 gram substance gave 40.3 c.c. N at 14.0°C . and 761.0 mm. pressure = 17.13 per cent. N.
- V. 0.3970 gram substance gave after fusion with $\text{KOH} + \text{KNO}_3$ 0.0146 gram BaSO_4 = 0.50 per cent. S.
- VI. 0.3307 gram substance gave 0.0036 gram ash = 1.08 per cent.

Percentage composition of the ash-free substance.

						Average
C	49.18	49.29	—	—	—	49.23
H	6.83	6.85	—	—	—	6.84
N	—	—	17.47	17.32	—	17.40
S	—	—	—	—	0.51	0.51
O						26.02
						100.00

Here, again, there is no noticeable difference in composition between the deutero body and the original gelatin.

In reactions, deuterogelatoze differs from the proto body mainly in not being precipitated at all by saturation of its aqueous solution with salt, or by addition of acetic acid to the salt-saturated fluid.

Further, with platinic chloride no precipitate at all is produced, or only a very slight one. In other respects there is no marked difference to be observed between the two gelatoses, except that the deutero body gives a less pronounced precipitate with mercuric chloride, and on boiling with Millon's reagent there is less of a coagulum to be observed; simply a pink colour to the solution.

Treatment of gelatin with alkaline trypsin solution.

By long-continued treatment of gelatin with an active trypsin solution considerable true peptone is formed, but in every case a large proportion of gelatoses will be found associated with the peptone. Under ordinary circumstances, however, where the ferment solution is not too active, the gelatoses predominate and the amount of true peptone formed is comparatively small.

Two distinct digestions were made, but a description of one will suffice to make clear the method of procedure and the results.

600 grams of pure, partially dried gelatin were placed in 2½ litres of 0.5 per cent. sodium carbonate and 250 c.c. of a pure, dialyzed trypsin solution, prepared according to Kühne's method, added, and the mixture, well thymolized, warmed at 40° C. for one week. A small residue remained undissolved, resembling in appearance the antialbumid-like matter noticed in the gastric digestion. The clear, limpid fluid containing the products of digestion was exactly neutralized, without producing any precipitate, although addition of acid in slight excess to a small portion caused a decided turbidity. The neutral fluid was concentrated to a thick syrup, without sign of gelatinization, and while

hot treated with a mixture of 95 per cent. alcohol and absolute alcohol, by which a thick brownish gum was thrown down. The alcoholic filtrate, after evaporation, on being tested with bromine water failed to give any characteristic colour, neither could any decisive reaction for tyrosin be obtained.

The precipitate produced by alcohol was dissolved in hot water and the solution saturated with ammonium sulphate, by which a heavy gummy precipitate of gelatoses was obtained, composed of a mixture of the two bodies, proto and deuto. The filtrate gave decided reactions for true peptone.

The ammonium sulphate precipitate was dissolved in water, the solution reprecipitated hot with ammonium sulphate, and the resultant mass of gelatoses treated in exactly the same manner as already described under gastric digestion, excepting that each final product was repeatedly boiled with strong alcohol to remove any possible adhering traces of crystalline bodies.

A certain amount of protogelatose was precipitated by simple saturation of the neutral fluid with salt, as in the gastric digestion, but as the dried product, after dialysis, etc., contained 8 per cent. of ash it was not further studied. Pure protogelatose was separated in exactly the same manner as described under the head of gastric digestion, viz. by addition of acetic acid to the salt-saturated fluid. The reactions of the body were essentially the same as those already described. The composition was likewise found to be the same, after drying at 110°C ., as shown by the following results:

Protogelatose, pancreatic digestion.

- I. 0.3036 gram substance gave 0.1775 gram H_2O = 6.49 per cent. H and 0.5422 gram CO_2 = 48.70 per cent. C.
- II. 0.3028 gram substance gave 0.1769 gram H_2O = 6.49 per cent. H and 0.5387 gram CO_2 = 48.48 per cent. C.
- III. 0.2677 gram substance gave 40.2 c.c. N at 20.1°C . and 755.4 mm. pressure = 17.46 per cent. N.
- IV. 0.2639 gram substance gave 38.9 c.c. N at 19.7°C . and 772.4 mm. pressure = 17.55 per cent. N.
- V. 0.4367 gram substance fused with $\text{KOH} + \text{KNO}_3$ gave 0.0193 gram BaSO_4 = 0.60 per cent. S.
- VI. 0.5143 gram substance fused with $\text{KOH} + \text{KNO}_3$ gave 0.0190 gram BaSO_4 = 0.50 per cent. S.
- VII. 0.6060 gram substance gave 0.0106 gram ash = 1.74 per cent.
- VIII. 0.5480 gram substance gave 0.0097 gram ash = 1.77 per cent.

Percentage composition of the ash-free substance.

							Average
C	49.56	49.34	—	—	—	—	49.45
H	6.61	6.61	—	—	—	—	6.61
N	—	—	17.77	17.86	—	—	17.81
S	—	—	—	—	0.62	0.52	0.57
O							25.56
							100.00

Deutergelatoses, formed by the action of trypsin, was separated as described under the head of gastric digestion, and was found to have essentially the same reactions and composition as the similar body formed by the action of pepsin-acid.

Deutergelatoses, pancreatic digestion.

- I. 0.2631 gram substance gave 0.1581 gram H_2O = 6.67 per cent. H and 0.4684 gram CO_2 = 48.51 per cent. C.
- II. 0.2631 gram substance gave 0.1543 gram H_2O = 6.51 per cent. H and 0.4687 gram CO_2 = 48.58 per cent. C.
- III. 0.2823 gram substance gave 41.1 c.c. N at 20.0° C. and 772.5 mm. pressure = 17.31 per cent. N.
- IV. 0.2683 gram substance gave 39.1 c.c. N at 20.4° C. and 770.9 mm. pressure = 17.27 per cent. N.
- V. 0.5667 gram substance fused with KOH + KNO_3 gave 0.0288 gram $BaSO_4$ = 0.69 per cent. S.
- VI. 0.5108 gram substance fused with KOH + KNO_3 gave 0.0231 gram $BaSO_4$ = 0.62 per cent. S.
- VII. 0.5575 gram substance gave 0.0059 gram ash = 1.06 per cent.
- VIII. 0.5110 gram substance gave 0.0057 gram ash = 1.11 per cent.

Percentage composition of the ash-free substance.

							Average
C	49.03	49.10	—	—	—	—	49.07
H	6.74	6.58	—	—	—	—	6.66
N	—	—	17.54	17.50	—	—	17.52
S	—	—	—	—	0.69	0.62	0.65
O							26.10
							100.00

Another series of products obtained from a second pancreatic digestion showed substantially the same composition as the ones described.

Comparison of the several gelatoses analyzed shows that they all

have the same chemical composition and that they do not differ in composition from gelatin itself.

	Gelatin.	Gastric digestion.		Pancreatic digestion.	
		Proto-gelatose.	Deutero-gelatose.	Proto-gelatose.	Deutero-gelatose.
C	49.38	49.98	49.23	49.45	49.07
H	6.81	6.78	6.84	6.61	6.66
N	17.97	17.86	17.40	17.81	17.52
S	0.71	0.52	0.51	0.57	0.65
O	25.13	24.86	26.02	25.56	26.10
Ash	1.26	1.98	1.08	1.75	1.08

From the foregoing results it is evident that in the digestion of gelatin with gastric and pancreatic juice there are formed at least three distinct products. Of these, two are primary products, and these are the same whether formed by the action of pepsin or of trypsin. They are to be distinguished from the third product, gelatin-peptone, by being precipitated by saturation with ammonium sulphate, whereas the peptone proper is not affected by this salt, either in neutral or acid solutions. Further, the true peptone is not precipitated by either saturation with salt, or with salt and acetic acid. The primary products by further ferment action are gradually changed into true peptone, the proto passing first through the stage of deutero, thus making a close analogy with the changes known to occur in the digestion of the proteids proper. Owing to the difficulty in separating the ammonium sulphate completely from the peptone, we have not been able to obtain the latter in sufficient quantity and in a sufficient state of purity for analysis.

The gelatoses are readily soluble in cold water, are slowly diffusible, and in composition resemble gelatin itself. They are undoubtedly formed by hydration, but their chemical composition affords no evidence of this view.

Protogelatose differs from deuterogelatose by being partially precipitated on saturation of its neutral solution with salt, and completely precipitated by addition of a little acetic acid to the salt-saturated fluid. Deuterogelatose, on the other hand, is not affected by either of these reagents. Saturation of its aqueous or slightly acid solution with ammonium sulphate, however, will quickly and completely precipitate it. Further, protogelatose yields a heavy precipitate with chloroplatinic acid, soluble on heating, while pure deuterogelatose gives little or no precipitate with this reagent.

No trace of any body corresponding to heteroalbumose was observed.

